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Additional data collected during this study are available in "Fate and transport of nitrapyrin in agroecosystems: Occurrence in agricultural soils subsurface drains, and receiving streams in the Midwestern US," Woodward et al. (2019).

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List of Abbreviations

AMPA	Aaminomethylphosphonic acid
DI	Deionized
FMOC-Cl	9-Fluorenylmethyl chloroformate
GC	Gas chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LODs	Limits of detection
LOQs	Limits of quantification
QA/QC	Quality assurance and quality control
RSD	Relative standard deviation
SPE	Solid phase extraction
US	United States
USEPA	United States Environmental Protection Agency

Abstract

Agricultural fields in the Midwestern United States are commonly tile-drained. This drainage system is used to remove excess water from the soil profile to increase crop production and promote soil conservation. However, subsurface tile drainage can readily deliver nutrients and pesticides from agricultural fields into surrounding watersheds. Glyphosate is a widely used pesticide in Midwestern agricultural fields, especially for modified glyphosate-resistant soybean and corn cultivars. The goal of this project was to develop a reliable and accurate analytical method to monitor the occurrence of glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), in tile drain water and receiving river water collected in east central Illinois agricultural lands. In this study, an isotopic dilution method was developed to analyze trace levels of glyphosate and AMPA in water using liquid chromatography-tandem mass spectrometry (LC-MS/MS), combined with pre-column derivatization and solid phase extraction for sample preparation. The method recoveries of glyphosate and AMPA during the whole monitoring period ranged from 85 to 120% and 83 to 147%, respectively. The limit of detection of the developed methods for glyphosate and AMPA was 0.10 µg/L with a relative standard deviation (RSD) of < 10%. The developed method was used to monitor glyphosate and AMPA in tile drainage and their receiving watersheds (e.g., the Spoon River and Salt Fork). Glyphosate and AMPA were frequently detected in river water samples at concentrations ranging from 0.13 to 2.85 µg/L and 0.13 to 1.30 µg/L, respectively. In contrast, the occurrence and concentrations of glyphosate and AMPA in subsurface tile drainage were much less than those in the receiving waters. Study results suggest that surface runoff and soil erosion could be the major transport pathways for glyphosate and AMPA losses from agricultural fields to receiving surface water.

1. Introduction

1.1. Background

1.1.1. Glyphosate Use

Glyphosate [*N*-(phosphonomethyl)glycine] is a non-selective, post-emergence herbicide which was first patented and commercialized by the Monsanto Company in the 1970s (Franz et al., 1997). This herbicide exhibits a unique and effective biological mode of action by inhibiting the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase, a key component of the shikimate pathway of plant biosynthesis (Mesnage and Antoniou, 2017). Since 1996, glyphosate usage has increased exponentially with the introduction of genetically modified glyphosate-resistant soybean and corn cultivars (Duke and Powles, 2008). Currently, glyphosate is the most commonly used pesticide in the United States because of its effectiveness and low cost (Atwood and Paisley-Jones, 2017).

In comparison with conventional herbicides, glyphosate is assumed to pose a relatively lower risk to the environment and public health because: (1) it is considered to be only slightly toxic to mammals, birds, and most aquatic species and is not expected to bioaccumulate (Battaglin et al., 2005; Carlisle and Trevors, 1988); (2) it is rapidly degraded by microbial metabolisms producing aminomethylphosphonic acid (AMPA) (Rueppel et al., 1977); (3) the metabolite AMPA is less toxic than the parent herbicide (Giesy et al., 2000); and (4) it strongly sorbs to soil colloids, limiting its potential runoff and leaching from fields (Cerdeira and Duke, 2006, Coupe et al., 2012). Accordingly, the use of glyphosate has resulted in a significant reduction in many other herbicide applications to control weeds. For example, glyphosate use in nine U.S. Midwestern states increased by 120% from 1998 through 2002, while the uses of the conventional herbicides acetochor, alachlor, atrazine, cyanzaine, and metolachor decreased by 16, 78, 13, 100, and 56%, respectively (Battaglin et al., 2005).

1.1.2. Environmental Fate and Toxicity of Glyphosate

Like most conventional herbicides, glyphosate is typically applied to plant foliage by spraying, but it can accumulate in the soil from spray drift and wash-off from plant surfaces (Saunders and Pezeshki, 2015). Once in the soil, glyphosate is strongly adsorbed to soil minerals, especially iron (Fe) and aluminum (Al) oxides (Carlisle and Trevors, 1988). Glyphosate sorption to soils is similar to phosphate binding (Battaglin et al., 2005), suggesting that phosphate could compete with glyphosate for soil sorption sites (Gimsing et al., 2004). The glyphosate sorption capacity of soils can impact the herbicide's degradation. The half-life of glyphosate in surface water is reported to range from 7 to 14 days (Giesy et al., 2000), and its half-life in soils may vary from a few days to several months or even years, depending on the desorption rates of glyphosate bound to the soil surfaces (Borggaard and Gimsing, 2008; Ghafoor et al., 2011; Norgaard et al., 2014). A previous report has revealed an inverse correlation between the glyphosate degradation rate coefficient and glyphosate sorption capacity (Ghafoor et al., 2011). Although the main

degradation product AMPA has a lower toxicity compared with glyphosate, its half-life in the environment is greater than that of the parent herbicide (Grandcoin et al., 2017).

The extensive use of glyphosate currently raises concern for its residue accumulation in the environment and in agricultural products such as corn or soybeans. Glyphosate and its metabolite AMPA have been frequently detected in food (Gill et al., 2017), water (Battaglin et al., 2005; Coupe et al., 2012; Peruzzo et al., 2008), atmosphere (Battaglin et al., 2014; Chang et al., 2011), and even in human urine at levels around 1-10 μg/L (Niemann et al., 2015). For example, a biomonitoring study detected glyphosate in urine samples from 60% of farmers who applied this herbicide on their fields and from a small percentage of those farmers' spouses and children (Acquavella et al., 2004). Although the acute toxicity of glyphosate has been well characterized with the acceptable daily intake at 0.3 mg/kg bw/day, health risks arising from exposure to glyphosate at environmental levels are very controversial (Mesnage and Antoniou, 2017). Some studies indicate that long-term exposure to glyphosate is responsible for many chronic diseases (cancers, neuropathies, infections, and osteoporosis) (De Roos et al., 2005; Richard et al., 2005; Samsel and Seneff, 2013). In contrast, other publications have concluded that glyphosate is safe at levels below regulatory permissible limits (Kier, 2015; Kier and Kirkland, 2013). More interestingly, the regulatory limits in many countries are extremely varied. In the U.S., for example, the maximum contaminant limit for drinking water is 700 μg/L (USEPA, 2018). The recommended Canadian Drinking Water Guideline is 28 μg/L (Health Canada, 1987). In the European Union, the maximum permissible level for glyphosate in drinking water is 0.1 μg/L (Rubio et al., 2003), which is several orders of magnitude lower than that in the U.S.

1.1.3. Losses of Glyphosate from Agricultural Land

It is critical to reduce glyphosate losses to the surrounding watersheds from agricultural land because this herbicide is ubiquitous in the environment. In general, pesticide losses occur through spray drift, surface runoff and erosion, and discharge from subsurface drainage systems (e.g., tile drainage) (Brown and van Beinum, 2009; Sandin et al., 2018). Although glyphosate has no tendency to volatilize because of its low vapor pressure, it has been detected frequently in the atmosphere and water of U.S. Midwestern agricultural areas, either because of spray drift or wind erosion, especially during growing seasons (Battaglin et al., 2005; Chang et al., 2011). Manufacturers suggest that glyphosate has a low probability of losses from fields owing to its high affinity for soil particles and short half-life in soils. However, many field studies have concluded that glyphosate readily leaches and runs off from fields into receiving watersheds (Borggaard and Gimsing, 2008; Norgaard et al., 2014; Sandin et al., 2018; Saunders and Pezeshki, 2015; Shipitalo and Owens, 2011). Moreover, very few studies have explored glyphosate losses from agricultural fields through subsurface drainage systems (Sandin et al., 2018).

In the Midwestern U.S., subsurface tile-drain networks are widely used in most agricultural fields to redirect excess water. Subsurface tile drainage also alters soil structure and changes the existing hydrology, which can expedite the transport of nutrients, pesticides, and colloid-borne constituents (e.g., pathogens) through the soil profile into tile drains, and ultimately into nearby

watersheds (Gall et al., 2011; Kladivko et al., 2001). In general, glyphosate is considered to be immobile because it strongly adsorbs to soil particles. However, particle-facilitated transport could accelerate the leaching of glyphosate into subsurface drainage and ultimately groundwater and surface water resources. A soil column study showed that about 17 and 68% of the leached glyphosate from untilled and recently plowed soils, respectively, were particle bound (Gjettermann et al., 2009). We hypothesized that the particle-facilitated transport and subsurface drainage systems could accelerate glyphosate losses from agricultural lands. To date, knowledge about the fate, transport, and losses of glyphosate from tile-drained fields of the Midwestern U.S. is essentially nonexistent.

1.1.4. Analysis Methods for Glyphosate

Glyphosate analysis of environmental samples is a challenge because glyphosate is made up of highly polar, small molecules and has a structural similarity to many naturally occurring compounds such as amino acids (Battaglin et al., 2005; Rubio et al., 2003). Moreover, glyphosate is highly soluble in water, thereby making its extraction with organic solvents difficult. The enzyme-linked immunoassay technique is a convenient and direct method for analyzing glyphosate (Clegg et al., 1999; Rubio et al., 2003). However, the method lacks sufficient selectivity and also is prone to interferences caused by the presence of extract matrices and similar non-target compounds. Alternatively, gas chromatography (GC) or liquid chromatography (LC) coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) has been adopted frequently to quantify concentrations of glyphosate accurately (Borjesson and Torstensson, 2000; Steinborn et al., 2016; Yoshioka et al., 2011). To reach superior sensitivity and high selectivity, chromatographic methods usually require either precolumn or postcolumn derivatization (Gill et al., 2017; Moye and Deyrup, 1984; Sancho et al., 1996; Steinborn et al., 2016). Precolumn derivatization of glyphosate and AMPA with 9-fluorenylmethyl chloroformate (FMOC-Cl) can significantly improve their chromatographic separation and detection (Figure 1), which is appropriate to measure glyphosate and AMPA in surface water or groundwater samples because their concentration levels frequently lie in the range of ng/L to µg/L (Ghanem et al., 2007; Meyers et al., 2009).

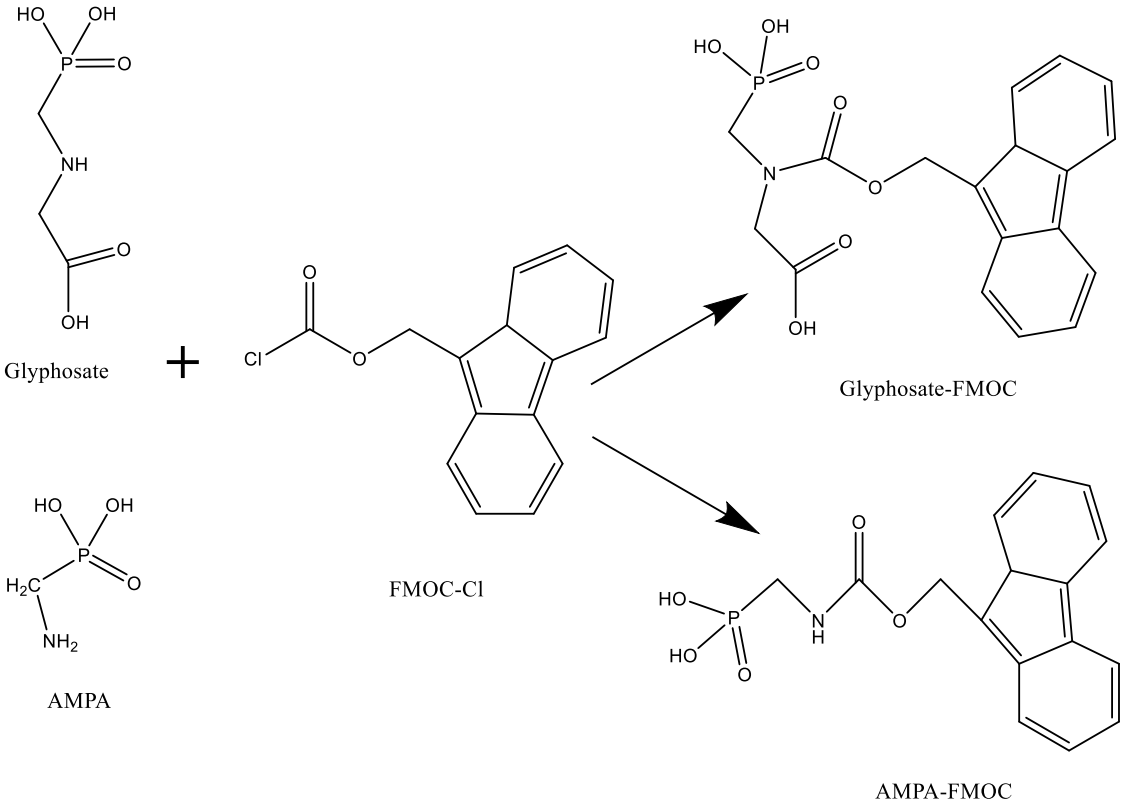

Figure 1. Derivative reactions of glyphosate and AMPA with FMOC-Cl.

1.2. Project Objective and Scope

The objective of this project was to investigate the occurrence of the herbicide glyphosate and its metabolite AMPA in subsurface tile drain water and receiving river water during a growing season in a tile drained agricultural area of east central Illinois, and thereby assess pesticide losses through subsurface tile drainage systems and identify the transport pathway. To achieve the goal, the following tasks were outlined for this project:

1) Develop and optimize analytical methods to measure glyphosate and AMPA in various water samples.

2) Investigate the spatial and temporal occurrence of glyphosate and AMPA in tile drainage water and receiving river water samples collected from east central Illinois agricultural lands.

3) Assess the losses of glyphosate and AMPA through subsurface tile drainage systems and identify their transport pathways from agricultural fields to nearby surface water.

2. Methodology

2.1. Chemicals and Materials

Glyphosate (99.5%) was purchased from Chem Service (West Chester, PA). AMPA (99%) and 9-FMOC-Cl (≥ 99.0%) were obtained from Sigma-Aldrich (St. Louis, MO). Isotope standards $^{13}C_2$-glyphosate and $^{13}C_2$-AMPA were purchased from Cambridge Isotope (Andover, MA) and Cerilliant Corporation (Round Rock, TX), respectively. All other chemicals used in the study, including sodium tetraborate, phosphoric acid, methanol, acetone, and acetonitrile, were of analytical grade and obtained from Fisher Scientific (Fair Lawn, NJ). Deionized (DI) water (> 18.0 MΩ-cm) was supplied by a Labconco Water Pro Plus system (Kansas City, MO). The solid phase extraction (SPE) cartridges (Waters Oasis HLB 6cc and HCB 3cc) were purchased from Waters Corporation (Milford, MA).

2.2. Field Sites and Water Sample Collection

In 2017, water samples including tile drainage, ditch, and river water were collected during the crop growing season in the Salt Fork and Spoon River watersheds of east central Illinois (Figure 2a). Major crops near the sampling sites were corn and soybeans. The Spoon River is a tributary of the Salt Fork and is located in Champaign County, IL. Six sampling sites are located near the Spoon River. Three tile drainage (SR02, SR03, and SR04) and two river water samples were collected underneath the bridges (SR01 and SR05) (Figure 2a and 2b). The Salt Fork is a tributary of the Vermilion River located in Illinois. In pre-settlement times, the Salt Fork drained a vast upland marsh. Presently, the Salt Fork has been extended by drainage ditches, and is about 70 miles long. There are eight sampling sites near the Salt Fork, including five tile drainage (SF02, SF03, SF04, SF05, and SF06) and two river water samples collected underneath the bridges (SF01 and SF07) (Figure 2a and 2c). Two field water runoff samples were also collected near sites SR06 and SF08. In addition, a ditch water sample near site SD01 was also collected (Figure 2a). For information online, click the following link:
https://www.google.com/maps/d/u/0/edit?hl=en&authuser=0&mid=1Ta9dDa4_M524_Dw74ln3TGYfZ5E&ll=40.16792264023891%2C-88.03450409999999&z=14

Water samples from the designed sites were collected weekly for three months (April through June 2017) by Illinois State Water Survey staff. The time of day was recorded following sampling. Water samples were collected in sterile wide-mouth glass bottles. Samples collected from bridge sites were depth integrated. Before sampling, the bottles were thoroughly rinsed with aliquots of the sample water prior to collection. All collected samples were immediately transported to the laboratory in an ice cooler. Note that not all tile drainage samples could be collected during each trip due to the absence of flow coming from the drains or flooding conditions submerging the outfall of the drain tiles. All samples were stored in a cold room (4 °C) at the Illinois Sustainable Technology Center and were derivatized within a week. A previous study showed

that concentrations of glyphosate and AMPA in underivatized water samples stored at 4 °C were relatively unchanged after 136 days (Meyers et al., 2009).

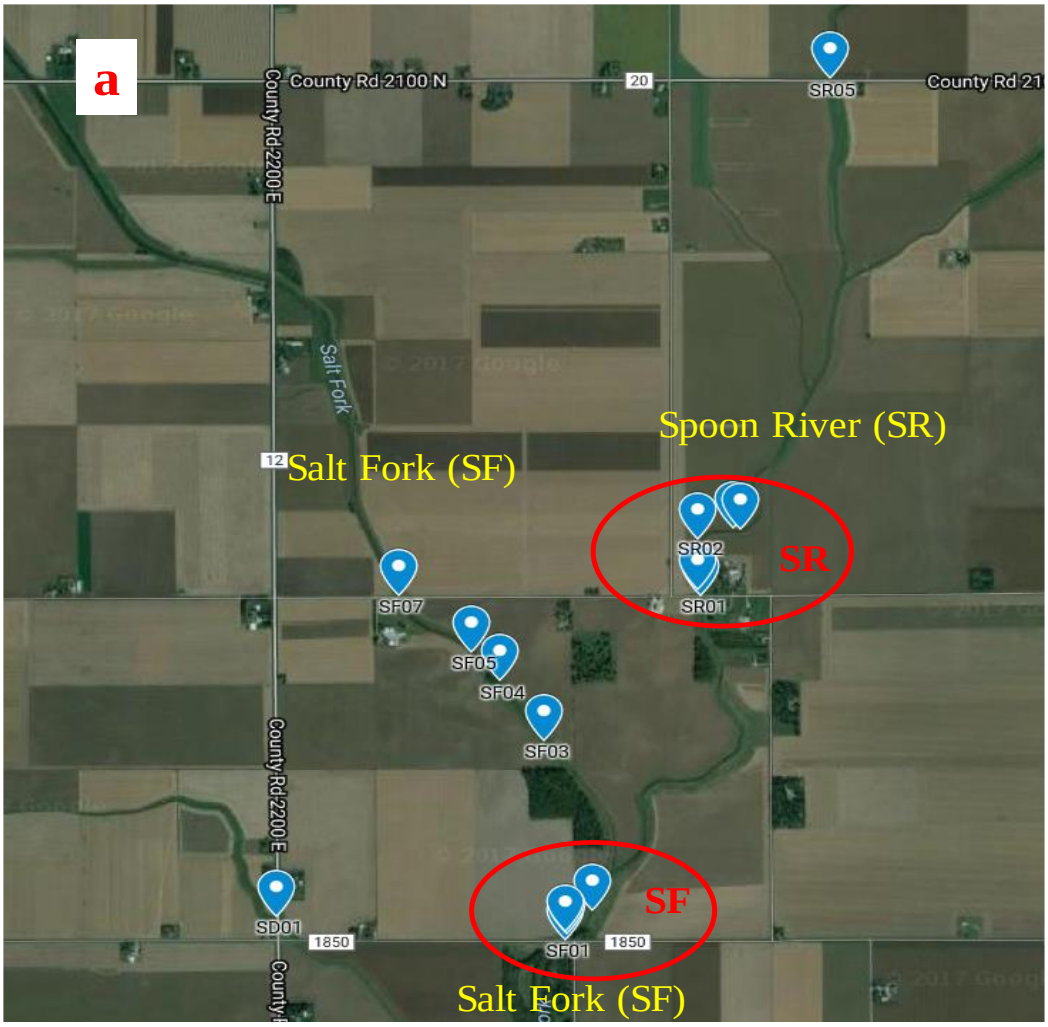

Figure 2a. Location of sampling sites along the Salt Fork (SF) and Spoon River (SR).

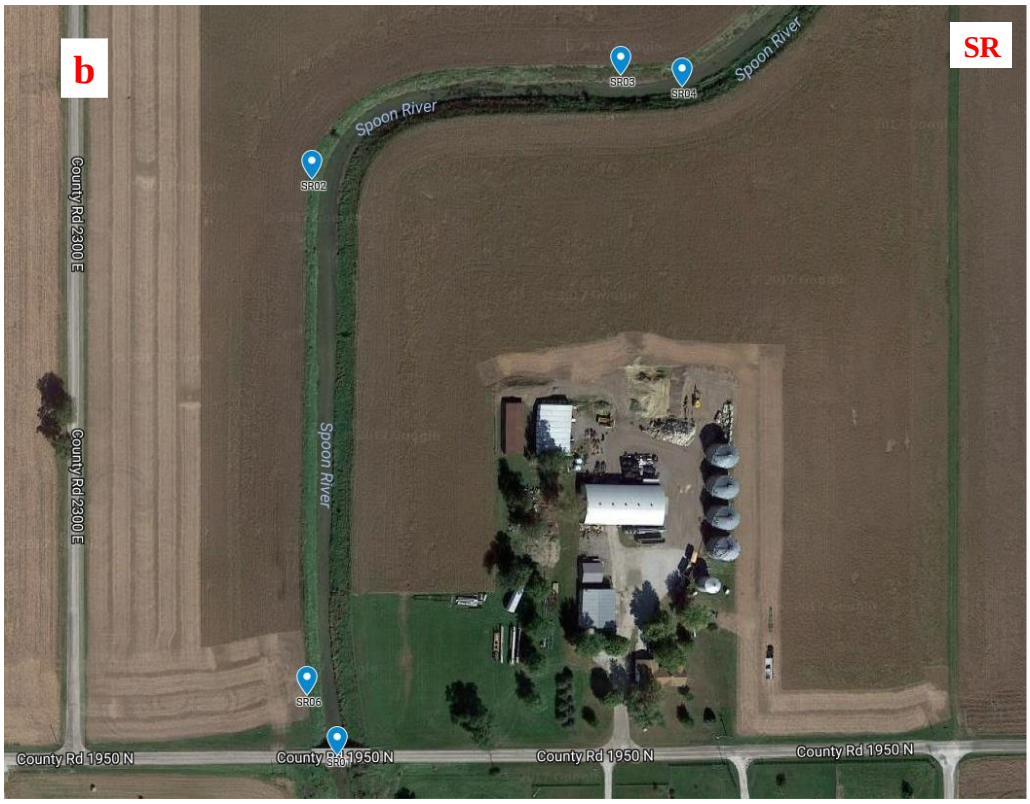

Figure 2b. Closer view of the red circled zones for Spoon River sampling sites.

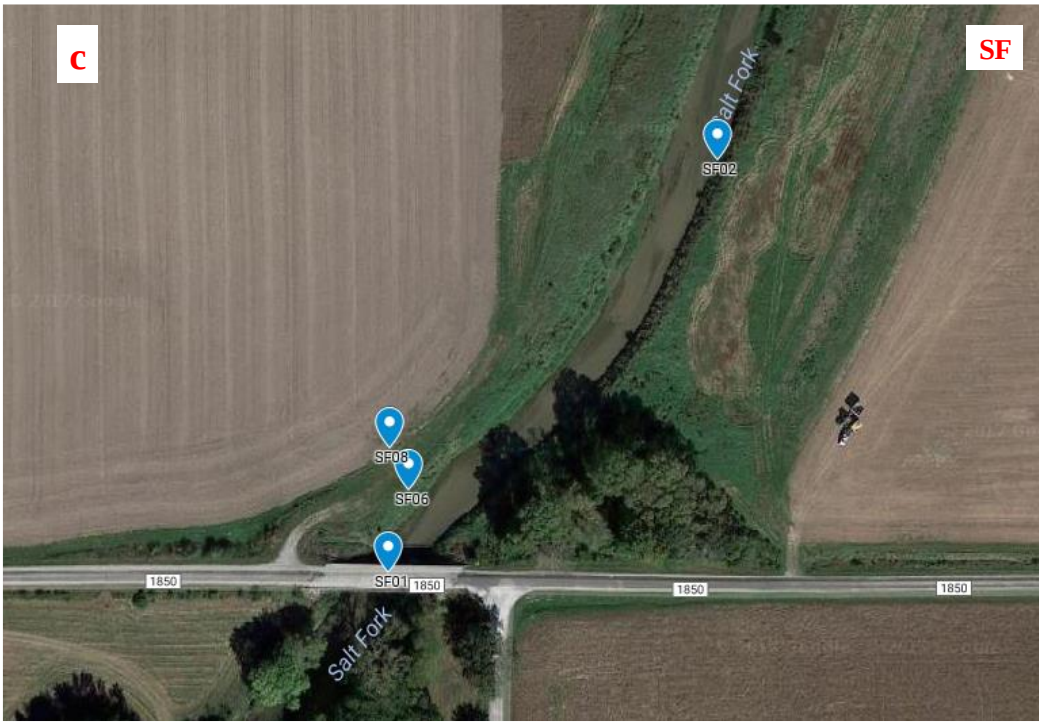

Figure 2c. Closer view of the red circled zones for Salt Fork sampling sites.

2.3. Sample Derivatization and Extraction

The water samples were derivatized based on a method proposed by Meyer et al. (2009), with some modifications. Briefly, 20 mL of water sample or blank water sample were placed into 25 mL screw-top glass tubes together with isotope-labeled glyphosate and AMPA (50 µg/L). All samples were adjusted to pH 9 by adding 0.5 mL of 5% sodium tetraborate buffer solutions. The samples were derivatized by adding 2.0 mL of FMOC (5 mM) in acetonitrile, vortexed for 1 min, and then incubated in a water bath. After derivatization, 0.6 mL of 2% phosphoric acid were added to each sample and vortexed for 1 min. In this study, six replications for each collected water sample were performed.

In addition, a preliminary experiment was performed to optimize the derivative process. Fortification of water for recovery experiments was performed by adding mixed standards of glyphosate and AMPA, as well as their isotope standards, into blank water samples to yield fortification levels at 0.1 mg/L and 1.0 mg/L for each chemical. After derivatization, the samples were extracted and analyzed by LC-MS/MS to evaluate the derivatization efficiencies under different conditions (e.g., derivatization temperature, time, and the presence of acetonitrile). In this task, eight replications for each condition were performed simultaneously.

2.4. Solid Phase Extraction (SPE)

After derivatization, glyphosate and AMPA in all collected water samples were extracted using SPE by a method developed by Ghanem et al. (2007), with some modifications. Before loading the samples, SPE cartridges were first preconditioned with 5 mL of methanol, 5 mL of DI water, and 5 mL of phosphate buffer solution in a series. The conditioning liquids were allowed to pass through the SPE cartridge by gravity. The derived water samples were passed through the cartridges with the aid of a vacuum at a rate of around 3-5 mL/min. The cartridges were then washed with 5 mL of DI water and then air dried for 30 min. The samples were eluted with 10 mL of methanol. The methanol extracts were evaporated until dry under a gentle nitrogen gas and reconstituted with 0.25 mL of acetonitrile. Six replications for each collected water sample were performed. In addition, a preliminary experiment was conducted to compare the extraction capacities of two kinds of SPE cartridges (Waters Oasis HLB 6cc and HLB 3cc).

2.5. LC-MS/MS Analysis

An analysis of glyphosate and AMPA was performed with a Waters Alliance 2695 separation module coupled to a Quattro Micro tandem mass spectrometer. Separation for each sample extract was achieved with a Waters Symmetry C18 analytical column (2.1 x 130 mm, 3.5 µm particle size) by using a gradient mobile phase consisting of 5 mM ammonium bicarbonate pH 9 (mobile phase A) and acetonitrile (mobile phase B). The gradient started with 80% mobile phase A and 20% mobile phase B, and ramped up to 10% mobile phase A and 90% mobile phase B linearly in 4 min. The gradient was changed back to 80% mobile phase A and 20% mobile phase B in 0.1 min and re-equilibrated for 3 min. The flow rate of the mobile phase was 0.2 mL/min. All

injections were performed at a 20 µL volume. Under these conditions, the retention times for glyphosate and AMPA were 1.37 and 1.78 min, respectively.

For glyphosate and AMPA quantitation, the mass spectrometer was operated in negative mode with a desolvation gas flow rate at 650 L/min. The operation parameters including collision energy and cone voltage were optimized using glyphosate and AMPA standards. Quantitative analysis was performed in the multiple reaction monitoring (MRM) transitions. Confirmation of glyphosate and AMPA in all water samples was based on MRM ion transitions as well as comparing the retention time of each peak to its corresponding isotopic standard.

2.6. Quality Assurance and Quality Control (QA/QC)

At the beginning of each assay, the instrument was calibrated over a range of 10 to 500 µg/L with reference materials obtained from a commercial vendor (Cambridge Isotope Laboratories). All water samples were quantified by a calibration standard curve containing six points. Like each sample, calibration standards were derivatized and then analyzed with the isotope standards. Calculation of the final results was performed according to the isotope dilution method. The isotope glyphosate and AMPA results were utilized as a surrogate to calculate the native glyphosate and AMPA final results in collected water samples. For each analysis batch, QA/QC consisted of at least one water blank, one duplicate spike-blank, and one triplicate sample. Triplicate sample results matched within 10% of each other, and glyphosate and AMPA were never detected in any water blanks during the study.

3. Results and Discussion

3.1. Development and Optimization of Analytical Methods for Glyphosate

3.1.1. Optimization of Derivatization of Glyphosate

Glyphosate and its main metabolite, AMPA, are highly polar, water soluble, and non-volatile compounds. They also lack fluorophore and chromophore groups, making their detection a challenge. In general, derivatization with chromophoric groups can make glyphosate and AMPA more volatile and improves their chromatographic separation and detection sensitivity. There are several derivatized reagents such as FMOC-Cl, *p*-toluenesulfonyl chloride, *p*-nitrobenzoyl chloride, (+)-1-(9-fluorenyl)ethyl chloroformate, trimethyl orthoacetate, and *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (Yoshioka et al., 2011). FMOC-Cl is an excellent reagent that converts glyphosate and AMPA to their FMOC derivatives (Figure 1), which yields highly informative fragment ions that allow definitive identification and quantification of analytes by using mass spectrometry (Ghanem et al., 2007).

In this study, we validated, modified, and optimized the derivatization conditions based on a U.S. Geological Survey method (Meyers et al., 2009). The results from the preliminary experiment suggested that the derivatization temperature, reaction time, and the presence of acetonitrile were of great importance for reproducibility. For eight replicate experiments with 0.1 mg/L and 1.0 mg/L of glyphosate and AMPA in water, respectively, coefficients of variation for the derivative yields were less than 15% when the derivatization was carried out at 40 °C for over 12 h. A previous report showed that the derivatization could be completed within 30 min at 30 °C (Kusters and Gerhartz, 2010). However, we found the use of a relatively low derivatization temperature (e.g., 30 °C) or less reaction time led to poor recovery and precision in the quantitative analysis of glyphosate and AMPA (Hanke et al., 2008). To maintain a high reproducibility of the analytical method, all collected water samples were derivatized at 40 °C for over 24 h. In addition, we found that the addition of acetonitrile would facilitate the derivatization reaction because FMOC-Cl is more readily soluble in acetonitrile than water. A previous report also indicated that 10% of acetonitrile was required to attain a complete transformation of glyphosate and AMPA to their derivative products (Hanke et al., 2008). Thus, we used acetonitrile to make up FMOC-Cl working solutions and thereby keeping the acetonitrile concentration in all derivatization solutions over 10%.

3.1.2. Optimization of SPE

In this study, we validated, modified, and optimized the SPE procedure based on a method proposed by Ghanem et al. (2007). Extraction efficiencies of Waters Oasis HLB 6cc (150 mg) and HLB 3cc (60 mg) cartridges were tested for glyphosate-FMOC and AMPA-FMOC derivatization solutions in a preliminary study. When 10 and 50 mL of derivatization solutions were loaded into the Oasis HLB 6cc cartridge, no derivatives were detected in the pass-through solutions. For the Oasis HLB 3cc cartridge, no derivatives were found when the loading volume of derivatization

solutions was 10 mL. However, when 50 mL of derivatization solutions passed through the cartridge, about 5% of glyphosate-FMOC was detected.

The volume of methanol used to elute glyphosate and AMPA derivatives was also tested. For the Oasis HLB 6cc cartridge, three 4 mL of methanol aliquots in a series were loaded into the cartridge and collected separately. There were no detectable derivatives in the last 4 mL of methanol elution solution. For the Oasis HLB 3 cc cartridge, three 3 mL of methanol aliquots in a series were used to elute the glyphosate and AMPA derivatives. No detectable derivatives were found in the last 3 mL of methanol elution solution. This preliminary study suggested that the elution methanol volumes were 8 mL for Oasis HLB 6cc and 6 mL for Oasis HLB 3cc cartridges. In this study, we used Oasis HLB 6cc cartridges to extract all collected water samples and 10 mL of methanol for elution.

3.1.3. Optimization of LC-MS/MS Conditions

Glyphosate and AMPA derivatives were analyzed and recorded in electrospray negative modes, which is a commonly used ionization method for glyphosate analysis (Meyers et al., 2009). Although some previous reports showed a better sensitivity for glyphosate and AMPA analysis by using a positive ion mode (Ghanem et al., 2007; Yoshioka et al., 2011), we did not observe that in this study. The MS/MS operation conditions were optimized by directly infusing standard glyphosate and AMPA derivatives as well as their isotopic standards. To optimize the cone voltage, the variations of current ion intensities were explored for both glyphosate and AMPA derivatives in $[M - H]^-$ ions (negative ionization). The results showed the optimum values of the cone voltage were 10 V and 30 V for glyphosate and AMPA, respectively (Table 1). To optimize the collision voltage, the fragmentation of $[M - H]^-$ ions was investigated as a function of collision energy. Optimum values of the collision voltage were 5 and 15 V for glyphosate and AMPA derivatives, respectively (Table 1).

Table 1. Retention times and optimized MS/MS parameters for glyphosate and AMPA derivatives.

Analyte	RT (min)	Ion model	MRM ions	Cone (V)	Collision (V)
Glyphosate-FMOC	1.37	-	389.84→167.75	10	5
$^{13}C_2$-glyphosate-FMOC	1.37	-	391.90→170.00	10	5
AMPA-FMOC	1.78	-	331.80→109.79	30	15
$^{13}C_2$-AMPA-FMOC	1.78	-	335.80→114.00	30	15

The representative MRM chromatograms in the negative mode to quantify glyphosate and AMPA derivatives as well as their isotopic standards (100 µg/L for each) are shown in Figure 3. The results clearly suggest that the developed analytical method is able to detect glyphosate and AMPA in a very short time (< 2.5 min). For the MS/MS analysis, two ion transitions (parent ion → product ion) for quantitation and confirmation are usually chosen for each analyte according to intensity and specificity criteria for applying the MRM mode. In this study, the MRM transitions of 389.84→167.75 and 331.80→109.79 were used to quantify glyphosate and AMPA, respectively (Table 1).

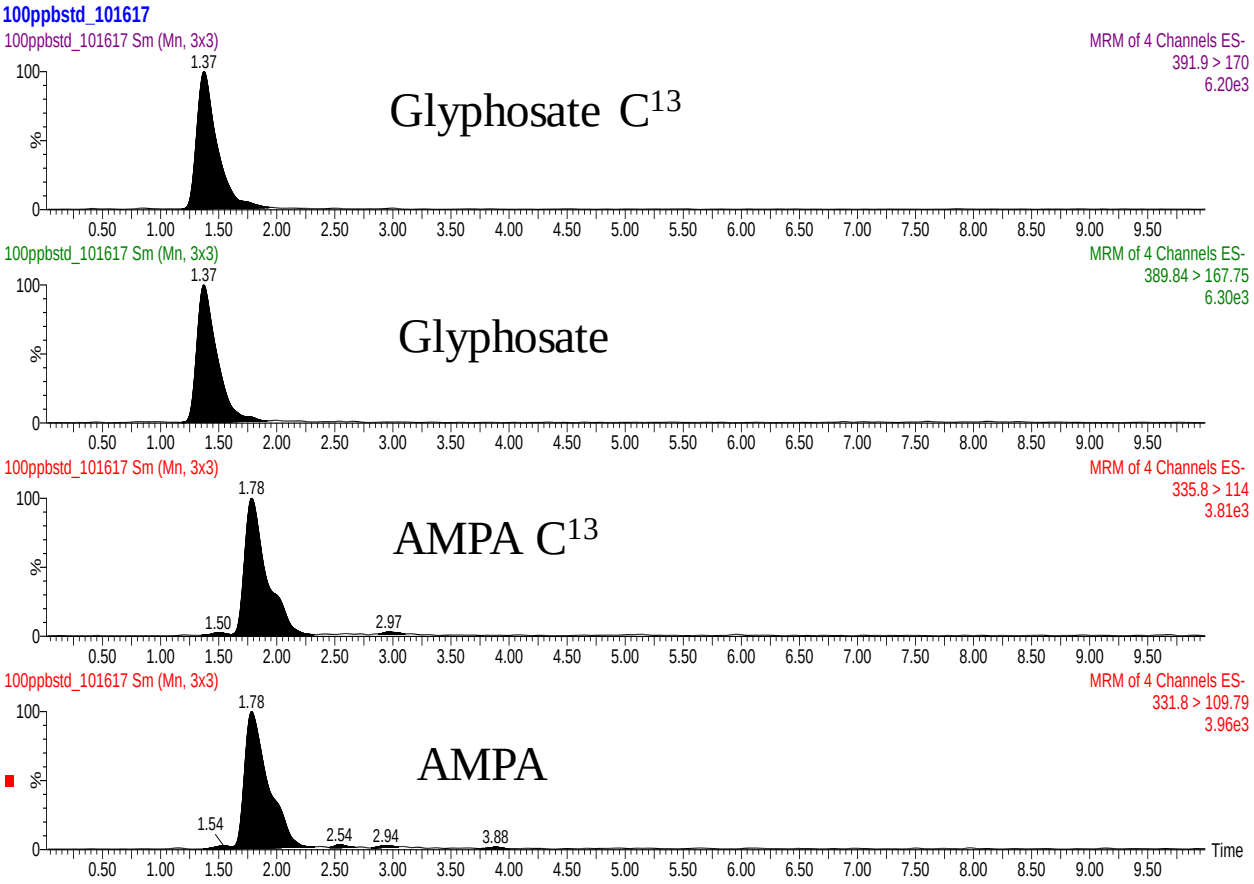

Figure 3. MRM chromatograms in the negative mode for quantitation of glyphosate and AMPA derivatives as well as their isotopic standards.

3.2. Method Validation

The entire procedure used to detect and quantify concentrations of glyphosate and AMPA in the collected water samples included isotope standard addition, derivatization with FMOC-Cl, extraction using an SPE cartridge, and quantification by LC-MS/MS. The performance of the entire developed method was evaluated by considering response linearity, recoveries, and limits of detection (LODs) of each targeted analyte in water samples. For analyte quantification, six-point calibration curves were performed for each targeted compound. A relative response was calculated with the standards using the ratios of the integrated peak areas for each targeted analyte and its corresponding isotope surrogate (USEPA, 2007). Good linearity was achieved for standard calibration of all compounds, with squared Pearson coefficients (R^2) > 0.99.

To determine the recovery of the developed method, the water samples were spiked with glyphosate and AMPA as well as their isotopic standards (1.0 mg/L for each). Glyphosate and AMPA isotopic standards are made with ^{13}C and ^{15}N, isotopes of ^{12}C and ^{14}N, respectively. Then the entire analytical procedure was applied. The results showed that the method has satisfactory recoveries for glyphosate (85-120%) and AMAP (83-147%). In addition, the developed method was found to be accurate and precise with the relative standard deviation (RSD) < 10% for both targeted compounds.

LODs for this method were calculated according to an established method (Vanderford and Snyder, 2006). Limits of quantification (LOQs) were used as reporting limits in this study and were calculated as three times the corresponding LODs. In this method, the LODs for glyphosate and AMAP were 0.10 µg/L, which were similar to a previous study (Meyers et al., 2009). The relatively low LODs render this method appropriate for the detection of trace levels of glyphosate and AMPA in water, and especially for monitoring the environmental samples. Reporting limits in this study were chosen to be greater than the LODs.

3.3. Occurrence and Fate of Glyphosate in Tile Drainage and Receiving Rivers

3.3.1. Occurrence of Glyphosate in the Spoon River and Salt Fork Watersheds

The developed method was applied to monitor the occurrence of glyphosate in the heavily agricultural Spoon River and Salt Fork watersheds where glyphosate is typically used. In the spring of 2017, river water from two targeted sites (SR01 and SF01) was collected weekly for three months (from April to June). Glyphosate was not detected in either river water sample in the beginning of the growing season (i.e., April 3, 2017), possibly because application of the herbicide had not yet occurred (Figures 4 and 5). This result also suggests that carryover from the previous applications was negligible.

In the Spoon River, glyphosate was first detected on April 11, 2017 (Figure 4). The overall detection frequency is 85% (11 of 13 samples) (Figure 6) at concentrations ranging from 0.13 to 2.85 µg/L. In the Salt Fork, glyphosate was first detected on May 1 (Figure 5), with an overall

detection frequency of 69% (9 of 13 samples) (Figure 6) and concentrations ranging from 0.14 to 2.85 μg/L. Because these two rivers are surrounded by cornfields, frequent detections of glyphosate in the river water samples indicate that the herbicide is widely used in these two watersheds and some is available for off-field transport.

In central Illinois, spring corn planting occurs roughly between mid-April and mid-May, depending on spring weather conditions. The glyphosate is usually applied between early April and late June. Figures 4 and 5 showed a similar temporal pattern for the occurrence of glyphosate in the Spoon River and Salt Fork. There are two pulses of glyphosate concentrations in both rivers. The first high concentrations of glyphosate were observed after a storm event on May 1, 2017, when both river flows were near maximum (Figures 4 and 5). This flush of glyphosate into nearby surface water during spring rainfall events is a typical phenomenon, which often has been observed for many other herbicides such as atrazine, acetochlor, and metolachlor in the Midwest (Thurman et al., 1991, Woodward et al., 2016). The second highest glyphosate concentrations were detected on June 14, 2017, possibly attributed to the glyphosate application because no rainfall happened during that time.

3.3.2. Occurrence of Glyphosate in Subsurface Tile Drainage

In this study, eight subsurface drainage tiles, which discharge excess field water into the Spoon River or Salt Fork, were selected for three-month monitoring for the occurrence of glyphosate. Surprisingly, glyphosate was detected in only three of the eight subsurface tile drainages, including twice in sites SF02 and SF05 (15% detection frequency) and once in site SR02 (8% detection frequency) during the entire monitoring period (Figure 6). Moreover, glyphosate concentrations in the tile drainage waters were relatively low compared with concentrations in the river water samples, ranging from 0.11 to 0.44 μg/L. These results contrasted with our hypothesis and previous reports, which indicated that glyphosate was readily transported through soils via preferential flow paths and thereby reached subsurface drainage and groundwater (Norgaard et al., 2014; Sandin et al., 2018).

3.3.3. Transport Pathways of Glyphosate Losses to Receiving River from Subsurface Drainage

In agricultural watersheds, glyphosate can enter surface waters through both surface runoff and subsurface flow. In Illinois agricultural fields with subsurface drainage, much of the subsurface flow is conveyed by tile drains directly to receiving streams or rivers. Thus, our initial hypothesis was that the subsurface title drainage could be one of the significant pathways for glyphosate losses from agricultural fields because of the wide application of this herbicide and the extensive adoption of drainage systems in Illinois. However, the overall detection frequency of glyphosate from tile drainage is only 5% (5 of 104 samples), which is much less than its occurrence in the receiving rivers.

To ensure that the occurrence of glyphosate in the Spoon River or Salt Fork was derived from the targeted agricultural areas, water samples upstream of the targeted areas (SR05 for Spoon River;

SF07 for the Salt Fork) were also collected for analysis. With one exception (May 22 Spoon River sample), glyphosate was not detected in upstream water samples. This result suggests that glyphosate in the two targeted rivers is mostly derived from the surrounding fields. Losses of glyphosate via subsurface tile drainage are not significant, and surface runoff could be a major transport pathway for glyphosate losses to the surface water. In addition, no drainage or water runoff occurred at the end of June because of drought. However, high concentrations of glyphosate were observed in both river water samples (Figures 4 and 5), suggesting that soil erosion could be an important mechanism to cause herbicide losses because of its strong sorption capacity in surface soils.

3.3.4. Occurrence of AMPA in the Subsurface Drainage and Receiving Rivers

Glyphosate in the environment is degraded to AMPA by microbial metabolism. This degradation product AMPA is structurally similar to the parent herbicide and can be further transformed into carbon dioxide and ammonium. However, the half-life of AMPA is believed to be greater than that of glyphosate in soils (Chang et al., 2011). AMPA and glyphosate are often found together. During the three-month monitoring period of this study, AMPA was frequently detected in the Spoon River or Salt Fork, with the detection frequency of 85% and 77%, respectively. The detected AMPA concentrations ranged from 0.13 to 1.30 μg/L, which were a little lower than glyphosate concentrations in most of the river water samples (Figures 4 and 5). Similarly, AMPA was detected in only three of the eight subsurface tile drainages, including twice in site SF02 (15% detection frequency) and once in sites SR02 and SR03 (8% detection frequency) during the entire monitoring period (Figure 7). These results imply that AMPA has similar transport pathways as its parent herbicide glyphosate.

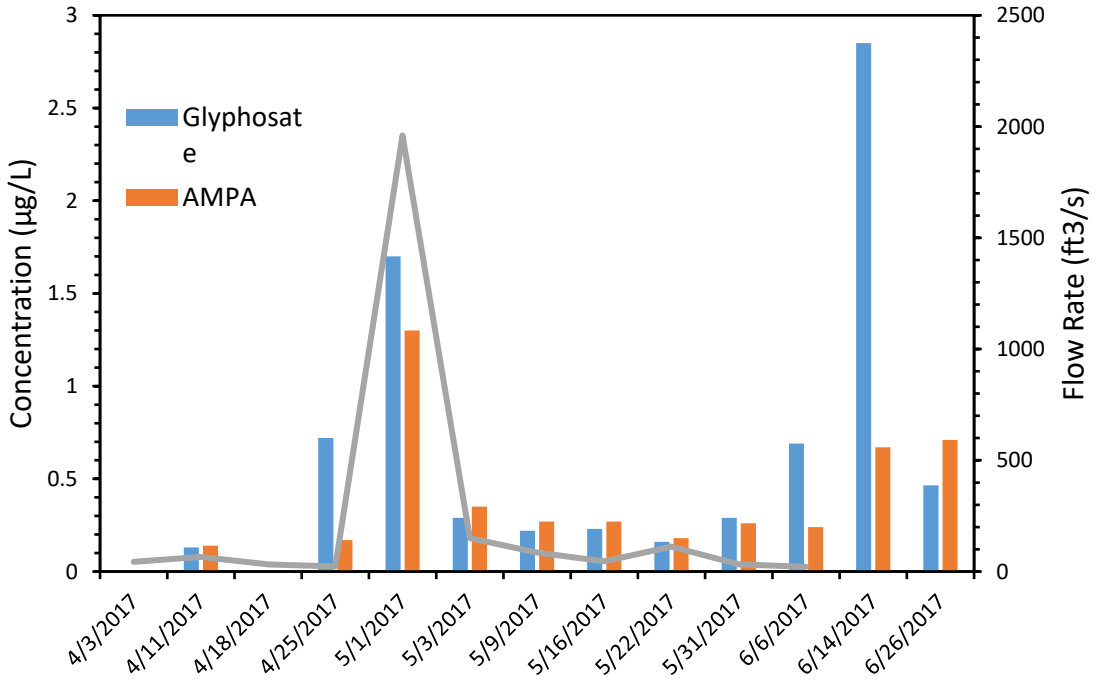

Figure 4. Concentrations of glyphosate and AMPA as well as the river flow for the Spoon River at site SR01, April-June 2017.

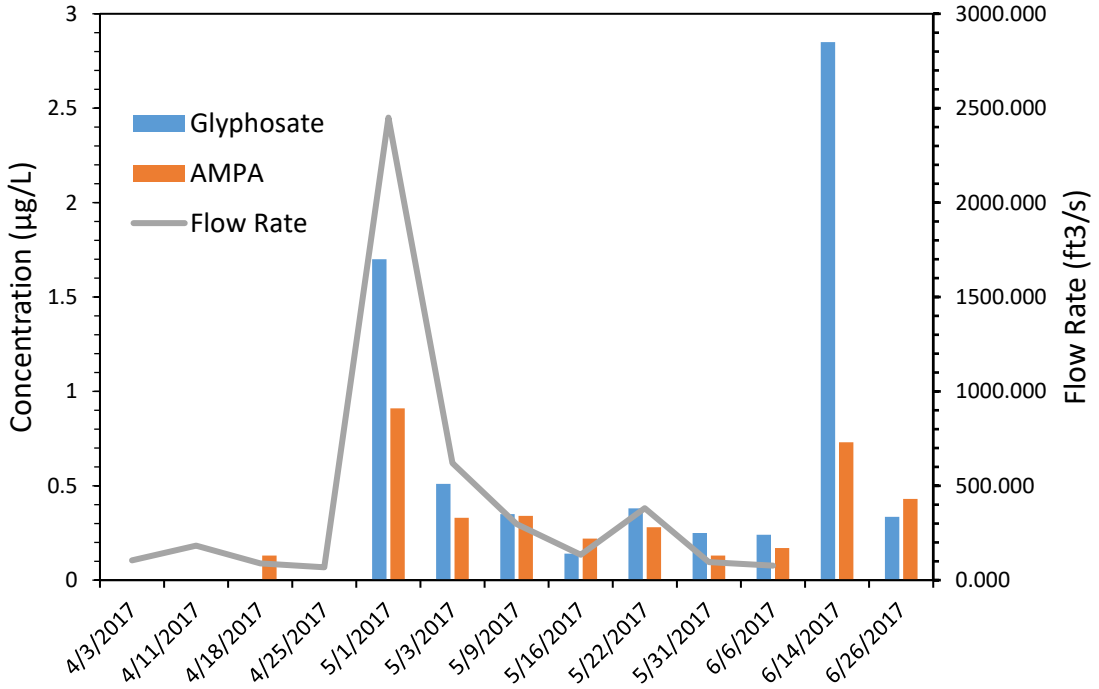

Figure 5. Concentrations of glyphosate and AMPA as well as the river flow for the Salt Fork at site SF01, April-June 2017.

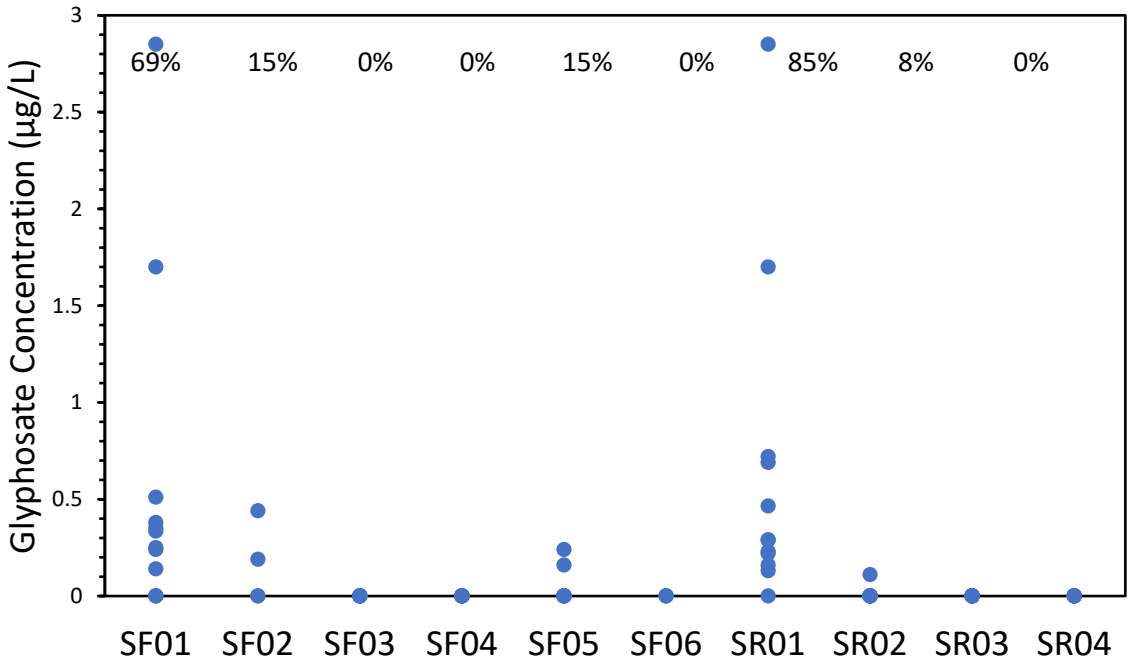

Figure 6. The detection frequency of glyphosate as well as its concentrations for the two river sites and eight tile drainage sites. The detection frequency is listed across the top.

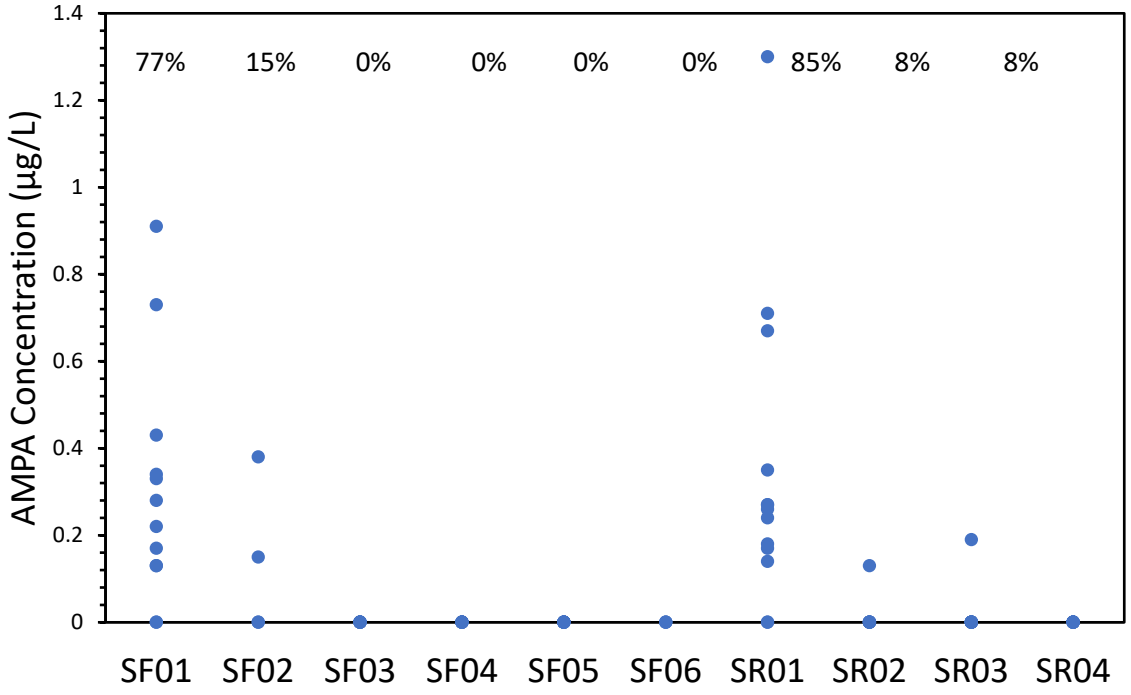

Figure 7. The detection frequency of AMPA as well as its concentrations for the two river sites and eight tile drainage sites. The detection frequency is listed across the top.

4. Conclusions

In this project, we developed and optimized precolumn derivatization, SPE, and LC-MS/MS methods for trace analysis of glyphosate and AMPA in water. To overcome matrix effects, stable isotopically labeled standards were added as internal standards. LODs of the developed methods for glyphosate and AMPA were 0.10 μg/L, which were sufficient for monitoring water samples collected from tile drainage and rivers.

Using the developed method, we conducted a monitoring study to investigate the occurrence of the herbicide glyphosate and its metabolite AMPA in tile drainage water and receiving rivers across east central Illinois agricultural lands during the spring and early summer of 2017. Our initial hypothesis was that both soil particle-facilitated transport and subsurface drainage systems could accelerate glyphosate losses from agricultural lands. However, this hypothesis was only partially supported by the monitoring results. Glyphosate and AMPA were frequently detected in river water samples collected from the Salt Fork and Spoon River. In contrast, the occurrence and concentrations of glyphosate and AMPA in subsurface tile drainage were much lower than those in the receiving rivers. The results from this study suggest that surface runoff and soil erosion could be major transport pathways for glyphosate and AMPA losses to the surrounding surface water during our study.

5. Recommendations

This project aimed to develop an accurate and efficient method for the simultaneous monitoring of glyphosate and AMPA in subsurface tile drainage and receiving river water. In addition, the purpose of this project was to provide some preliminary data concerning the occurrence of glyphosate in the environment, including possible transport pathways from fields to nearby watersheds. This preliminary monitoring work was conducted in a small area for a short period (three months) because of limited funding. Thus, the monitoring results have a certain contingency and may not represent the typical fate and transport of glyphosate in tile-drained fields. The results from this project indicate that the losses of glyphosate via subsurface drainage are not significant, and surface runoff could be a major transport pathway for the herbicide losses. In this project, we also conducted a simple runoff study and attempted to validate the main transport pathway of glyphosate from the fields. However, glyphosate and AMPA were not detected in two runoff water samples collected at sites SR06 and SF08. Thus, a more extensive runoff study is necessary to identify accurately the transport pathway of glyphosate in tile-drained fields.

To assess glyphosate comprehensively and accurately identify its transport pathways, a long-term monitoring study is a necessity. There are several recommendations for further studies:

- Conduct a relatively long-term monitoring study (e.g., a five-year monitoring program).
- Extend the monitoring period from growing to harvest seasons (e.g., from April to September).

- Simultaneously investigate phosphorus transport. Establish the possible relationship between glyphosate and phosphorus losses since they have a similar binding property on soils.
- Conduct a more detailed runoff study to ascertain the main pathways of glyphosate losses.

6. References

Acquavella, J.F., B.H. Alexander, J.S. Mandel, C. Gustin, B. Baker, P. Chapman, and M. Bleeke. 2004. Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. *Environ. Health Perspect.* 112: 321-326. doi:10.1289/ehp.6667.

Atwood, D., and C. Paisley-Jones. 2017. *US EPA - Pesticides Industry Sales and Usage 2008 - 2012*. U.S. Environmental Protection Agency, Washington, DC.

Battaglin, W.A., D.W. Kolpin, E.A. Scribner, K.M. Kuivila, and M.W. Sandstrom. 2005. Glyphosate, other herbicides, and transformation products in Midwestern streams, 2002. *J. Am. Water Resour. Assoc.* 41: 323-332. doi:10.1111/j.1752-1688.2005.tb03738.x.

Battaglin, W.A., M.T. Meyers, K.M. Kuivila, and J.E. Dietze. 2014. Glyphosate and its degradation product AMPA occur frequently and widely in US soils, surface water, groundwater, and precipitation (1). *J. Am. Water Resour. Assoc.* 50: 275-290. doi:10.1111/jawr.12159.

Borggaard, O.K., and A.L. Gimsing. 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review. *Pest Manag. Sci.* 64: 441-456. doi:10.1002/ps.1512.

Borjesson, E., and L. Torstensson. 2000. New methods for determination of glyphosate and (aminomethyl) phosphonic acid in water and soil. *J. Chromatogr.* A 886: 207-216. doi:10.1016/s0021-9673(00)00514-8.

Brown, C.D., and W. van Beinum. 2009. Pesticide transport via sub-surface drains in Europe. *Environ. Pollut.* 157: 3314-3324. doi:10.1016/j.envpol.2009.06.029.

Carlisle, S.M., and J.T. Trevors. 1988. Glyphosate in the environment. *Water Air Soil Pollut.* 39: 409-420.

Cerdeira, A.L., and S.O. Duke. 2006. The current status and environmental impacts of glyphosate-resistant crops: A review. *J. Environ. Qual.* 35: 1633-1658. doi:10.2134/jeq2005.0378.

Chang, F.C., M.F. Simcik, and P.D. Capel. 2011. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ. Toxicol. Chem.* 30: 548-555. doi:10.1002/etc.431.

Clegg, B.S., G.R. Stephenson, and J.C. Hall. 1999. Development of an enzyme-linked immunosorbent assay for the detection of glyphosate. *J. Agric. Food Chem.* 47: 5031-5037. doi:10.1021/jf990064x.

Coupe, R.H., S.J. Kalkhoff, P.D. Capel, and C. Gregoire. 2012. Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. *Pest Manag. Sci.* 68: 16-30. doi:10.1002/ps.2212.

De Roos, A.J., A. Blair, J.A. Rusiecki, J.A. Hoppin, M. Svec, M. Dosemeci, D.P. Sandler, and M.C. Alavanja. 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. *Environ. Health Perspect.* 113: 49-54.

Duke, S.O., and S.B. Powles. 2008. Glyphosate: a once-in-a-century herbicide. *Pest Manag. Sci.* 64: 319-325. doi:10.1002/ps.1518.

Franz, J.E., M.K. Mao, and J.A. Sikorski. 1997. *Glyphosate: A Unique Global Herbicide*. American Chemical Society, Washington, DC.

Gall, H.E., S.A. Sassman, L.S. Lee, and C.T. Jafvert. 2011. Hormone discharges from a Midwest tile-drained agroecosystem receiving animal wastes. *Environ. Sci. Technol.* 45: 8755-8764. doi:10.1021/es2011435.

Ghafoor, A., N.J. Jarvis, T. Thierfelder, and J. Stenstrom. 2011. Measurements and modeling of pesticide persistence in soil at the catchment scale. *Sci. Total Environ.* 409: 1900-1908. doi:10.1016/j.scitotenv.2011.01.049.

Ghanem, A., P. Bados, L. Kerhoas, J. Dubroca, and J. Einhorn. 2007. Glyphosate and AMPA analysis in sewage sludge by LC-ESI-MS/MS after FMOC derivatization on strong anion-exchange resin as solid support. *Anal. Chem.* 79: 3794-3801. doi:10.1021/ac062195k.

Giesy, J.P., S. Dobson, and K.R. Solomon. 2000. Ecotoxicological Risk Assessment for Roundup (R) Herbicide. In *Reviews of Environmental Contamination and Toxicology*, ed. G.W. Ware, 167: 35-120. Springer, New York.

Gill, J.P.K., N. Sethi, and A. Mohan. 2017. Analysis of the glyphosate herbicide in water, soil and food using derivatising agents. *Environ. Chem. Lett.* 15: 85-100. doi:10.1007/s10311-016-0585-z.

Gimsing, A.L., O.K. Borggaard, and P. Sestoft. 2004. Modeling the kinetics of the competitive adsorption and desorption of glyphosate and phosphate on goethite and gibbsite and in soils. *Environ. Sci. Technol.* 38: 1718-1722. doi:10.1021/es030572u.

Gjettermann, B., C.T. Petersen, C.B. Koch, N.H. Spliid, C. Gron, D.L. Baun, and M. Styczen. 2009. Particle-facilitated pesticide leaching from differently structured soil monoliths. *J. Environ. Qual.* 38: 2382-2393. doi:10.2134/jeq2008.0417.

Grandcoin, A., S. Piel, and E. Baures. 2017. AminoMethylPhosphonic acid (AMPA) in natural waters: Its sources, behavior and environmental fate. *Water Res.* 117: 187-197. doi:10.1016/j.watres.2017.03.055.

Hanke, I., H. Singer, and J. Hollender. 2008. Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: Performance tuning of derivatization, enrichment and detection. *Anal. Bioanal. Chem*. 391: 2265-2276. doi:10.1007/s00216-008-2134-5.

Health Canada. 1987. *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Glyphosate*.Kier, L.D. 2015. Review of genotoxicity biomonitoring studies of glyphosate-based formulations. *Crit. Rev. Toxicol*. 45: 209-218. doi:10.3109/10408444.2015.1010194.

Kier, L.D., and D.J. Kirkland. 2013. Review of genotoxicity studies of glyphosate and glyphosate-based formulations. *Crit. Rev. Toxicol.* 43: 283-315. doi:10.3109/10408444.2013.770820.

Kladivko, E.J., L.C. Brown, and J.L. Baker. 2001. Pesticide transport to subsurface tile drains in humid regions of North America. *Crit. Rev. Environ. Sci. Technol.* 31: 1-62. doi:10.1080/20016491089163.

Kusters, M., and M. Gerhartz. 2010. Enrichment and low-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in drinking water after cleanup by cation exchange resin. *J. Sep. Sci.* 33: 1139-1146. doi:10.1002/jssc.200900556.

Mesnage, R., and M.N. Antoniou. 2017. Facts and fallacies in the debate on glyphosate toxicity. *Front Public Health* 5. doi:Unsp 31610.3389/Fpubh.2017.00316.

Meyers, M.T., K.A. Loftin, E.A. Lee, G.H. Hinshaw, J.E. Dietze, and E.A. Scribner. 2009. *Determination of Glyphosate, its Degradation Product Aminomethylphosphonic Acid, and Glufosinate, in Water by Isotope Dilution and Online Solid-Phase Extraction and Liquiquid*

Chromatography/Tandem Mass Spectrometry. Techniques and Methods 5-A10. U.S. Geological Survery.

Moye, H.A., and C.L. Deyrup. 1984. A simple single-step derivatization method for the gas-chromatographic analysis of the herbicide glyphosate and its metabolite. *J. Agric. Food Chem.* 32: 192-195. doi:10.1021/jf00122a005.

Niemann, L., C. Sieke, R. Pfeil, and R. Solecki. 2015. A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. *J. Verbrauch. Lebensm.* 10: 3-12. doi:10.1007/s00003-014-0927-3.

Norgaard, T., P. Moldrup, T.P.A. Ferre, P. Olsen, A.E. Rosenbom, and L.W. de Jonge. 2014. Leaching of glyphosate and aminomethylphosphonic acid from an agricultural field over a twelve-year period. *Vadose Zone J.* 13. doi:10.2136/vzj2014.05.0054.

Peruzzo, P.J., A.A. Porta, and A.E. Ronco. 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. *Environ. Pollut.* 156: 61-66. doi:10.1016/j.envpol.2008.01.015.

Richard, S., S. Moslemi, H. Sipahutar, N. Benachour, and G.E. Seralini. 2005. Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environ. Health Perspect.* 113: 716-720. doi:10.1289/ehp.7728.

Rubio, F., L.J. Veldhuis, B.S. Clegg, J.R. Fleeker, and J.C. Hall. 2003. Comparison of a direct ELISA and an HPLC method for glyphosate determinations in water. *J. Agric. Food Chem.* 51: 691-696. doi:10.1021/jf020761g.

Rueppel, M.L., B.B. Brightwell, J. Schaefer, and J.T. Marvel. 1977. Metabolism and degradation of glyphosate in soil and water. *J. Agric. Food Chem.* 25: 517-528. doi:10.1021/jf60211a018.

Samsel, A., and S. Seneff. 2013. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. *Entropy* 15: 1416-1463. doi:10.3390/e15041416.

Sancho, J.V., F. Hernandez, F.J. Lopez, E.A. Hogendoorn, E. Dijkman, and P. vanZoonen. 1996. Rapid determination of glufosinate, glyphosate and aminomethylphosphonic acid in environmental water samples using precolumn fluorogenic labeling and coupled-column liquid chromatography. *J. Chromatogr.* A 737: 75-83. doi:10.1016/0021-9673(96)00071-4.

Sandin, M., K. Piikki, N. Jarvis, M. Larsbo, K. Bishop, and J. Kreuger. 2018. Spatial and temporal patterns of pesticide concentrations in streamflow, drainage and runoff in a small Swedish agricultural catchment. *Sci. Total Environ.* 610: 623-634. doi:10.1016/j.scitotenv.2017.08.068.

Saunders, L.E., and R. Pezeshki. 2015. Glyphosate in runoff waters and in the root-zone: A Review. *Toxics* 3: 462-480. doi:10.3390/toxics3040462.

Shipitalo, M.J., and L.B. Owens. 2011. Comparative losses of glyphosate and selected residual herbicides in surface runoff from conservation-tilled watersheds planted with corn or soybean. *J. Environ. Qual.* 40: 1281-1289. doi:10.2134/jeq2010.0454.

Steinborn, A., L. Alder, B. Michalski, P. Zomer, P. Bendig, S.A. Martinez, H.G.J. Mol, T.J. Class, and N.C. Pinheiro. 2016. Determination of glyphosate levels in breast milk samples from Germany by LC-MS/MS and GC-MS/MS. *J. Agric. Food Chem.* 64: 1414-1421. doi:10.1021/acs.jafc.5b05852.

Thurman, E.M., D.A. Goolsby, M.T. Meyers, and D.W. Kolpin. 1991. Herbicides in surface waters of the Midwestern United States – The effect of spring flush. *Environ. Sci. Technol.* 25: 1794-1796. doi:10.1021/es00022a018.

USEPA. 2018. https://safewater.zendesk.com/hc/en-us/articles/212076457-4-What-are-EPA-s-drinking-water-regulations-for-glyphosate-.

USEPA. 2007. *Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS*.

Vanderford, B.J., Snyder, S.A., 2006. Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. *Environ. Sci. Technol*. 40, 7312–7320. doi:10.1021/es0613198

Woodward, E.E., M.L. Hladik, and D.W. Kolpin. 2016. Nitrapyrin in streams: The first study documenting off-field transport of a nitrogen stabilizer compound. *Environ. Sci. Technol. Lett.* 3: 387-392. doi:10.1021/acs.estlett.6b00348.

Woodward, E.E., D. W. Kolpin, W. Zheng, N. L. Holm, S. M. Meppelink, P. J. Terrio, M. L. Hladik. 2019. Fate and transport of nitrapyrin in agroecosystems: Occurrence in agricultural soils subsurface drains, and receiving streams in the Midwestern US. *Science of the Total Environment*. 650:2830-2841.

Yoshioka, N., M. Asano, A. Kuse, T. Mitsuhashi, Y. Nagasaki, and Y. Ueno. 2011. Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography-tandem mass spectrometry using hydrophilic interaction chromatography. *J. Chromatogr.* A 1218: 3675-3680. doi:10.1016/j.chroma.2011.04.021.